Figure 1

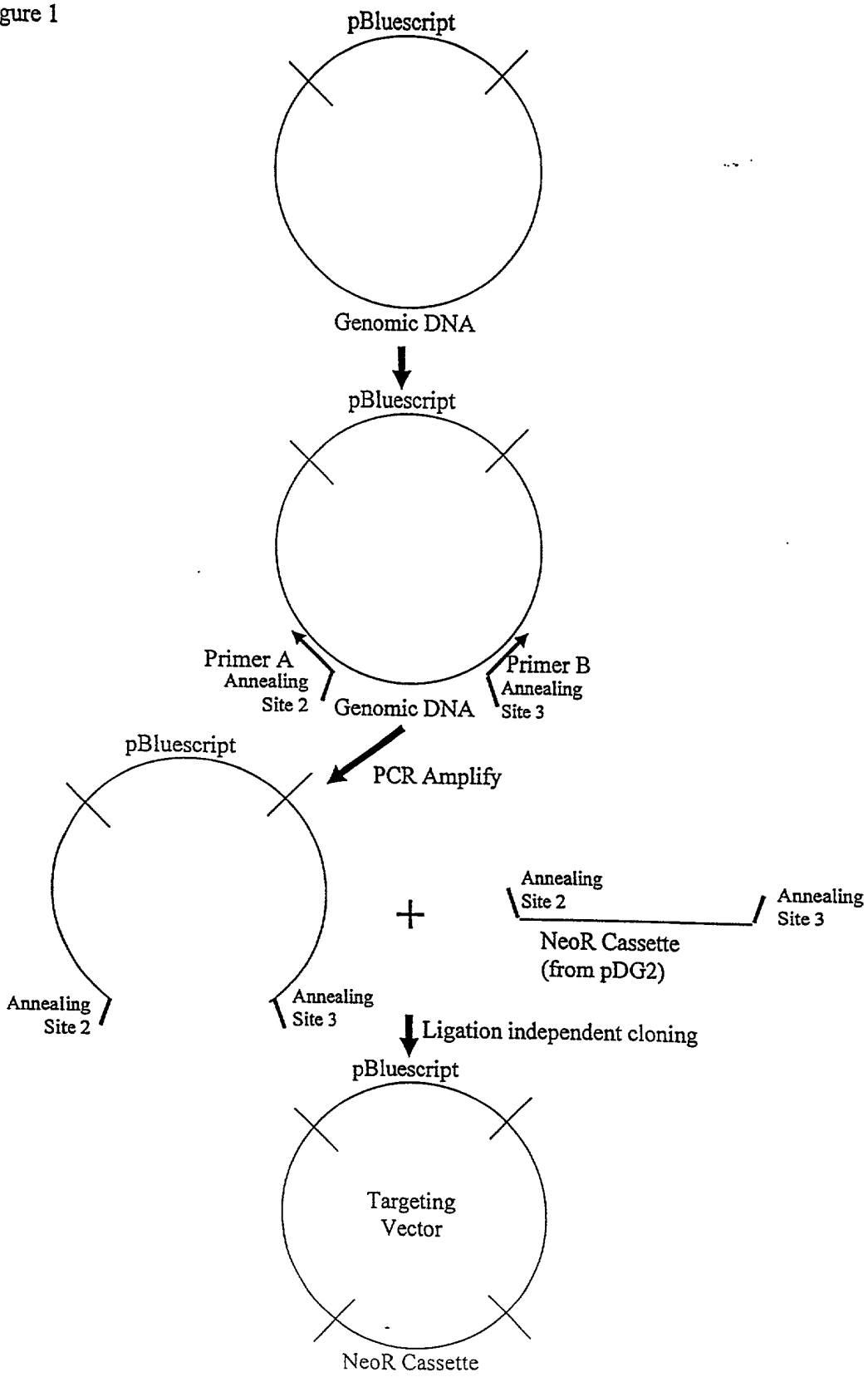
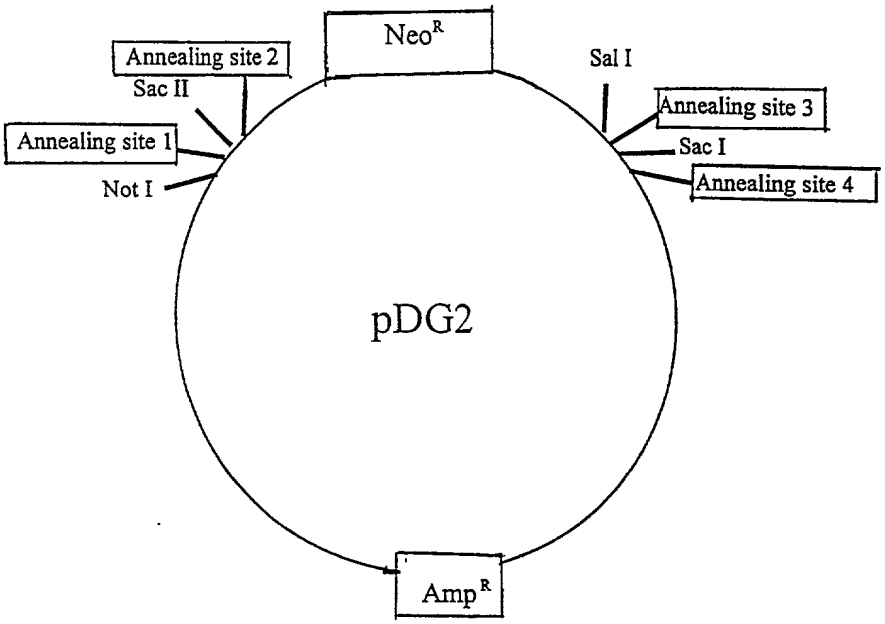

pBluescript
Genomic DNA
pBluescript
Primer A
Annealing
Site 2
Primer B
Annealing
Site 3
Genomic DNA
pBluescript
PCR Amplify
Annealing
Site 2
Annealing
Site 3
NeoR Cassette
(from pDG2)
Annealing
Site 2
Annealing
Site 3
Ligation independent cloning
pBluescript
Targeting
Vector
NeoR Cassette

Figure 2A



Plasmid Backbone

PDG2.SEQ.02202005

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Fig 2B

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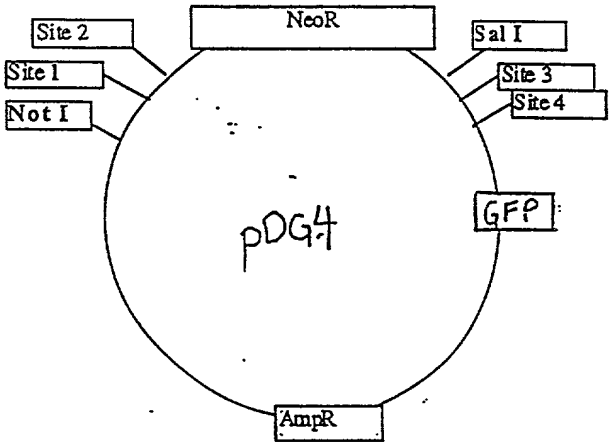

Fig 3A

pDG4:

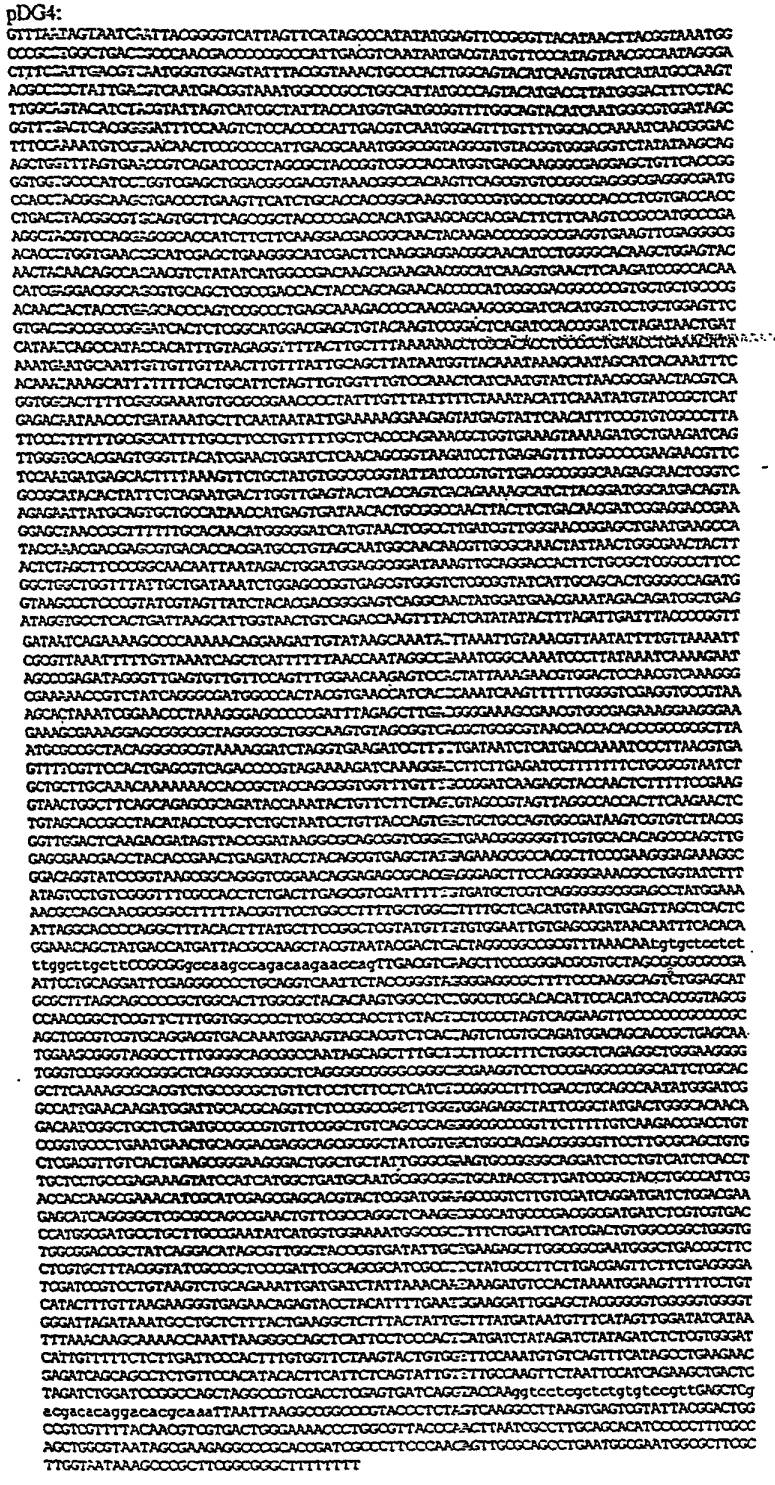

Fig 3B

Annealing site	Sequence	Sequence after digestion
1	5′ tgtgctcctctttggcttgcttccaa... 3′ 3′ acacgaggagaaaccgaacgaaggtt... 5′	5′ tgtgctcctctttggcttgcttccaa... 3′ 3′ tt... 5′
2	5′ ctggttcttgtctggcttggcccaa... 3′ 3′ gaccaagaacagaccgaacccgggtt... 5′	5′ ctggttcttgtctggcttggcccaa... 3′ 3′ tt... 5′
3	5′ ggtcctcgctctgtgtccgttgaa... 3′ 3′ ccaggagcgagacacaggcaactt... 5′	5′ ggtcctcgctctgtgtccgttgaa... 3′ 3′ tt... 5′
4	5′ tttgcgtgtcctgtgtcgtcgaa... 3′ 3′ aaacgcacaggacacagcagctt... 5′	5′ tttgcgtgtcctgtgtcgtcgaa... 3′ 3′ tt... 5′

Fig 4

EP 2 525 834 B1

Annealing site	Sequence	Sequence after digestion
1	5′ AAtgtgctcctctttggcttgcttCCGC 3′ 3′ Ttacacgaggagaaaccgaacgaagg 5′	5′ AA 3′ 3′ Ttacacgaggagaaaccgaacgaagg 5′
2	5′ AActggttcttgtctggcttggcCCGC 3′ 3′ Ttgaccaagaacagaccgaaccggg 5′	5′ AA 3′ 3′ Ttgaccaagaacagaccgaaccggg 5′
3	5′ AAggtcctcgctctgtgtccgttGAGCT 3′ 3′ Ttccaggagcgagacacaggcaac 5′	5′ AA 3′ 3′ Ttccaggagcgagacacaggcaac 5′
4	5′ AAtttgcgtgtcctgtgtcgtcGAGCT 3′ 3′ Ttaaacgcacaggacacagcagc 5′	5′ AA 3′ 3′ Ttaaacgcacaggacacagcagc 5′

Fig 5

EP 2 392 656 A1

EP 0 823 474 A2

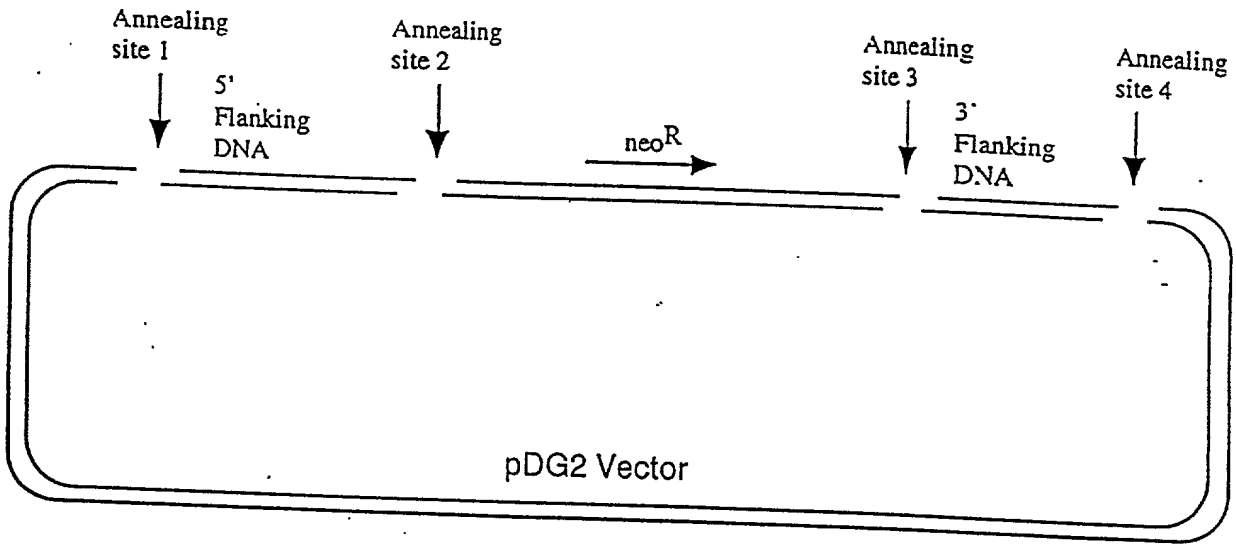

Fig 6

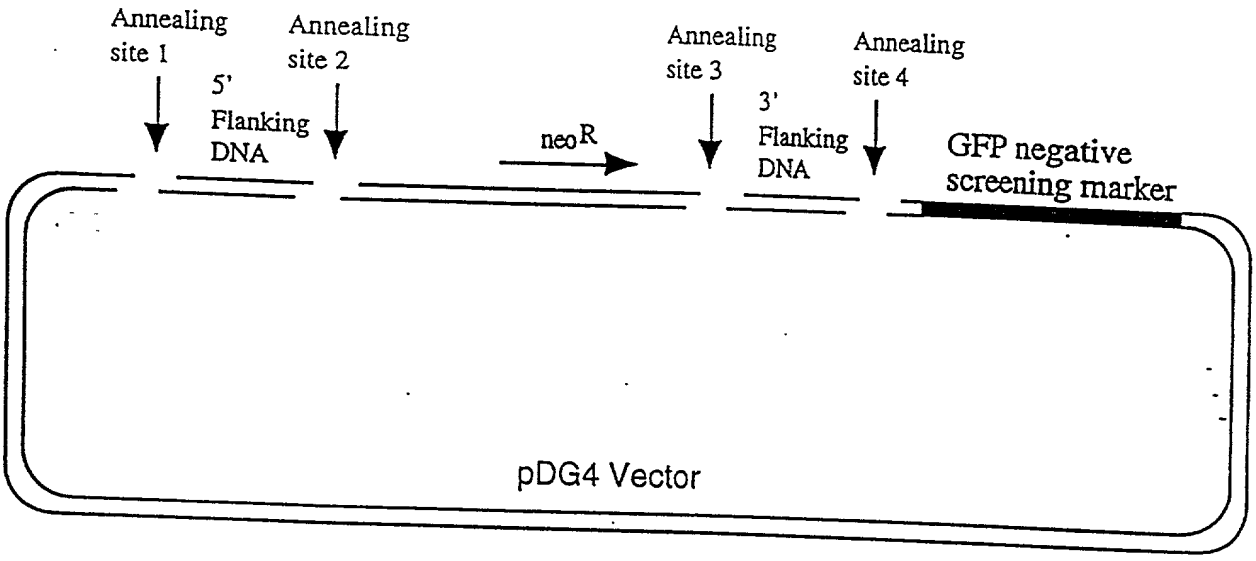

Fig 7

US 6,528,313 B1

Oligo#	Sequence (5′ to 3′)
174	ATGACCGCTCAGGAAACCTGTTGCA
180	ATAGGCATAGTAGGCCAGCTTGAGG
454	tgtgctcctctttggcttgcttccAATTAACCCTCACTAAAGGGAACGAAT
463	ctggttcttgtctggcttggcccaaTGCAACAGGTTTCCTGAGCGGTCAT
464	ggtcctcgctctgtgtccgttgaaCCTCAAGCTGGCCTACTATGCCTAT
42	tttgcgtgtcctgtgtcgtcgaaCGACTAATACGACTCACTATAGGGCG
151	GCCAATGGACTCTTAGTTTTGGAAC
155	GTTCTGGCAAACAAATTCGGCGCAC
454	tgtgctcctctttggcttgcttccAATTAACCCTCACTAAAGGGAACGAAT
465	ctggttcttgtctggcttggcccaaGTTCCAAAACTAAGAGTCCATTGGC
466	ggtcctcgctctgtgtccgttgaaGTGCGCCGAATTTGTTTGCCAGAAC
1	GAACCTTGGTGTGCCAAGTTACTTC
2	GAACTTTGGCTGAACCCCTTGTTCT
41	tgtgctcctctttggcttgcgttgaaCGACTAATACGACTCACTATAGGGCG
38	ctggttcttgtctggcttggcccaaGAAGTAACTTGGCACACCAAGGTTC
40	ggtcctcgctctgtgtccgttgaAGAACAAGGGGTTCAGCCAAAGTTC
37	tttgcgtgtcctgtgtcgtcgAATTAACCCTCACTAAAGGGAACGAAT
540	ATGCCGGATCTCCTACTACTGGGCC
546	TGTCATAGTAGACAGCGATGGAACG
445	GACAAGAACCAGTTGACGTCAAGCTTCCCGGGACGCGTGCTAGCGGCGCGCCG
667	ctggttcttgtctggcttggcccaaGGCCCAGTAGTAGGAGATCCGGCAT
668	ggtcctcgctctgtgtccgttgaaCGTTCCATCGCTGTCTACTATGACA
907	ctggttcttgtctggcttggcccaaAAAGCCGACAGCCACGCTCACAAGC
908	ggtcctcgctctgtgtccgttgaaGCCCAATGCCACAGAGACAGAATGT
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1158	ggtcctcgctctgtgtccgttgaaCTCCAGTGCCGAGTGTGTGGGGACAG

Express Mail No. EV 339 254 405 US

Figure 8